ON THE ACTION OF CERTAIN SUBSTANCES ON OXYGEN CONSUMPTION.

III. ACTION OF POTASSIUM CYANIDE ON SOME CŒLENTERATES AND ANNELIDS.

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In this paper are presented some further data concerning the effect of potassium cyanide on the rate of oxygen consumption of animals. In view of the importance of this substance as a reagent in physiological experiments, and since tests of this kind have been made upon comparatively few animals, it has seemed worth while to obtain data upon some representatives of groups in which the action of cyanide has not yet been determined. No experiments have, to my knowledge, been performed upon annelids, and only one cœlenterate has been tested, namely *Gonionemus*, in which form Loeb and Wasteneys ('13) showed that potassium cyanide decreases the oxygen consumption to a considerably greater extent than does ethyl urethane, although both produce the same degree of anæsthesia.

The literature dealing with the chemical, physiological and pharmacological properties of the cyanides and related substances has been extensively reviewed in the second paper of this series (Hyman, '19), and will therefore not be restated here. Briefly, it may be said that this group of substances has been shown to depress many physiological processes; and to decrease the rate of oxygen consumption or carbon dioxide output or both of the following living materials: yeast, a mould *Aspergillus*, a sponge *Suberites*, *Gonionemus*, *Planaria*, a beetle *Passalus cornutus*, embryos of *Fundulus*, several mammals, sea-urchin eggs, red blood corpuscles of geese, isolated mammalian kidneys, the frog's heart, minced beef liver, minced horse, beef, and pigeon muscle, and minced horse brain. In most of these cases it was shown that the effect was reversible. Since that review was

written, two additional papers have come to my attention, those of Allen ('19) and of Evans ('19). Allen's results and conclusions regarding the effect of potassium cyanide on the rate of oxygen consumption of *Planaria* are about the same as my own, except for one or two points; thus Allen states that the oxygen consumption of *Planaria* cannot be reduced to less than 20 per cent. of the normal by cyanide, while I found in many cases, reduction to 10 to 15 per cent. of the normal. Evans's interesting paper contains some further references to the literature, confirms some previous work, and presents a number of new facts. Thus Evans finds that cyanide decreases markedly and reversibly the oxygen consumption of the cat, confirms the older statements that oxygen is just as readily dissociable from the blood in cyanide poisoning as from normal blood; and shows a striking similarity between the behavior of smooth, skeletal, and heart muscle, and nerve and nervous centers in the presence of cyanide and in lack of oxygen. Evans concludes that the cyanides "appear to exert all of their physiological effects by reason of the anoxæmia which they produce," and favors the view that they act by uniting chemically with some tissue constituent.[1]

[1] Comment would seem to be required on Evans's remark (p. 23) that the observations of Lund and Herwerden seem to be incompatible with Child's conception of metabolic gradients. Lund's results have been considered elsewhere (Child, '19). Herwerden ('18) says that the buds and young of *Hydra* are not more susceptible to cyanide than the parents. Perusal of her paper shows, however, that she has compared only three or four pairs of individuals and has observed the *disintegration of the tentacles only*, having removed the cyanide when this occurred. Now, we have clearly shown (Child and Hyman, '19) that the buds of *Hydra* are not comparable with the parents *until they have the same degree of muscular activity*, and further that the susceptibility of the tentacles is also *dependent upon their degree of activity*. Miss Herwerden is mistaken in her conclusion. We have compared dozens of young and adult *Hydras*, belonging to three species, and have always found that the young are more susceptible to cyanide *when comparable degrees of muscular activity* exist. It is true that the difference is *least* in the case of the tentacles but even there the tentacles of the young are slightly more susceptible. Neither are Miss Herwerden's statements about the susceptibility of the Daphnid heart entirely correct. I have found that the smallest individuals in a *Daphnia* culture are the most susceptible of all (both as to heart and entire animal); that the susceptibility decreases with increasing size up through the size when the females are producing their first parthenogenetic broods; but from that time on, the relation is reversed, so that the largest and oldest individuals in the culture, which have produced many parthenogenetic broods, are more susceptible than

In the experiments to be reported in this paper it was not my purpose to make a complete study of the action of cyanide on oxygen consumption in these animals, since I had already done that in the case of a sponge and *Planaria*, but merely to show that cyanide in non-lethal doses reversibly decreases oxygen intake. The experiments on the marine forms were performed at the Marine Biological Laboratory, Woods Hole, Mass.; those on the fresh-water forms at the University of Chicago. I am greatly indebted to Professor F. R. Lillie for working space at the first-named institution.

The methods employed were identical with those used in previous experiments on this subject and will be found described in full in a former paper (Hyman, '19). Briefly, the animals to be tested were placed in Erlenmeyer flasks or wide-mouthed bottles of 500 to 600 c.c. capacity, these were filled air-tight with water, and the animals allowed to respire in them for a convenient length of time. A sample was then withdrawn, and this and a sample of the original water used were analyzed for oxygen content by Winkler's method, the difference between the two samples representing the oxygen consumed by the animals. Two separate determinations of the oxygen consumption in normal water were made, potassium cyanide was then added to the water and two more determinations in the presence of cyanide carried out. The animals were then washed in several changes of water, and their oxygen consumption in normal water tested again a day or two later. Throughout each experiment, the receptacles containing the animals were immersed in a water-bath, the temperature of which was kept constant to 0.5 degrees.

The possibility of iodine absorption by substances emanating from the organisms was again considered, and was tested in the case of two or three of the animals employed. Standard iodine solution was added to water which had been standing on the animals for at least one hour, and the iodine then titrated back

half-grown individuals. It would thus appear that in these animals parthenogenetic reproduction is accompanied by some degree of rejuvenescence. Green's recent work on *Simocephalus* (BIOL. BULL., Aug., 1919) supports this suggestion, since he found that these animals reproduce sexually early in ontogeny, and subsequently the same individuals begin parthenogenetic reproduction, a reversal of the order usually observed in parthenogenetic forms.

with thiosulphate. No loss of iodine was found, and indeed, this possible source of error in the Winkler method would seem to have been greatly overrated (*cf.* Hale and Melia, '13).

Experiments on Pennaria.

Pennaria tiarella, a common colonial hydroid of the Atlantic coast, was selected as a representative of this group of cœlenterates. Large quantities of freshly collected material were available. Clean branches, free from visible plant growth, were cut off, *Caprella* and other small animals which commonly live on such colonies carefully removed, and the branches were then placed in wide-mouthed bottles, and treated as described above. The animals were always used within a few hours after they were collected.

Table I.

Action of Potassium Cyanide on the Oxygen Consumption of *Pennaria tiarella.*

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Aug. 18, 19° C.			Aug. 23, 22° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.55	0.56	0.43	0.94	0.78	1.21
Second hour normal	0.62	0.56	0.43	0.95	0.74	1.20
	1/25000 Mol. KNC.			1/10000 Mol. KNC.		
First hour KNC	0.38	0.38	0.27	0.52	0.45	0.60
Second hour KNC	0.32	0.35	0.25	0.36	0.34	0.54
Per cent. decrease	41	35	39	54	49	53

Six different lots of *Pennaria* were tested in this manner, as to their normal rate of oxygen consumption and the rate in 1/10000 and 1/25000 mol. potassium cyanide. The results are given in Table I. In all cases, the rate of oxygen consumption is reduced in the presence of cyanide. Unfortunately in the case of this animal it could not be determined whether the effect was reversible, since *Pennaria* deteriorates rapidly in the laboratory; within twenty-four hours, the majority of the hydranths have either fallen off or have lost their normal appearance.

Since *Pennaria* exhibits very few movements, it is not possible

that differences in muscular activity could account for the decreased oxygen intake in the presence of cyanide. As far as could be determined, the hydranths were as fully expanded in the presence of cyanide as in normal sea-water.

Experiments on Metridium.

Metridium marginatum was selected as a representative of the Anthozoa. The individuals used had probably been kept in float cars for some time. Medium-sized individuals were placed in wide-mouthed bottles of about 600 c.c. capacity, one in each, and left there undisturbed until they had attached themselves and expanded fully. It was found that the animals soon became accustomed to such an environment and could be handled and would submit to change of water without contracting. Since the oxygen consumption of sea-anemones in all probability varies with the degree of expansion, note was always made of the degree of expansion during the experiments. In experiments 1 and 3, the animals were slightly contracted during the exposure to cyanide but in the other six experiments they were fully expanded throughout. Differences in state of muscular activity are therefore not responsible for the observed results.

The eight experiments which were performed upon *Metridium* are reported in Table II. A marked and reversible decrease in oxygen consumption in the presence of cyanide was found in all cases.

Table II.

Action of Potassium Cyanide on the Oxygen Consumption of *Metridium marginatum*.

No. of Experiment	1.	2.	3.	4.	5.	6.	7.	8.
Temp. and Date	Aug. 12, 21° C.			Aug. 10, 21.5° C.		Aug. 13, 20° C.		
Oxygen Consumed, Cubic Centimeters per Hour.								
First hour normal	1.33	1.19	1.13	1.66		1.06	1.01	0.97
Second hour normal	1.09	1.05	1.03	1.94	1.67	1.12	1.19	1.31
	1/5000 Mol. KNC.			1/10000 Mol.		1/25000 Mol.		
First hour KNC	0.48	0.56	0.56	1.24	1.25	0.81	0.70	0.66
Second hour KNC	0.49	0.59	0.42	0.89	0.70	0.70	0.76	0.59
Per cent. decrease	60	49	55	41	58	32	34	46
Recovery	1.09	1.10	1.14	1.68		1.08	1.18	1.20

Experiments on Polychætes.

Experiments were performed on three common polychæte annelids of the Atlantic coast—*Nereis virens*, *Arenicola cristata*, and *Chætopterus pergamentaceus*. Of the three species, *Chætopterus* was found to be the most favorable for this kind of experimentation, as it is not a very active animal. No difference in amount of activity in normal and cyanide-containing sea-water was observed. *Arenicola* was fairly favorable for the purpose as it also is relatively inactive, and here again there was no significant difference in activity throughout the experiments; indeed, it seemed to me that the respiratory movements were more pronounced in the presence of cyanide than in normal sea-water. On the other hand, the results with *Nereis* were not very satisfactory owing to the restlessness of the animals. In experiments 1–3, Table V, the animals were considerably more active in normal sea-water than they were after cyanide had been added, and hence the decrease observed in cyanide was due in part to diminished motor activity. In experiments 4–6, Table V., however, differences in motor activity were not present and have not contributed to the result.

A further difficulty was encountered in the case of *Nereis*. It was observed that both the degree of activity and the rate of oxygen consumption of *Nereis* diminished when it was kept in the laboratory. Experiments 1–3 were performed on freshly collected individuals and it will be noted that the oxygen consumption of these individuals is much higher than in the other cases, and further that the recovery figures, obtained upon them twenty-four hours later, are considerably lower than the original figures. This is not due to the exposure to cyanide since individuals from the same collection which had not been subjected to cyanide showed the same decrease in oxygen intake after a day in the laboratory. It is probable that this decrease is due in large part to starvation. I have observed it in the case of a number of other animals also, and my experience indicates that animals which have been kept for a few days after removal from their natural environment are preferable to freshly collected material for experiments of this kind. This difficulty was not encountered in the case of *Chætopterus* and *Arenicola* since it happened that

the animals had been on hand in the collecting department for some time before I obtained them.

Since the first experiments showed that some little time was required for the penetration of even relatively concentrated

TABLE III.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Chætopterus pergamentaceus.*

No. of Experiment	1.	2.	3.	4.	5.	6.	7.	8.	6.
Temp. and Date	Aug 3, 20.5° C.			Aug. 3, 21° C.			Aug. 5, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.									
First hour normal	0.48	0.40	0.56	0.44	0.36	0.45	0.47	0.35	0.44
Second hour normal	0.33	0.40	0.48	0.40	0.36	0.44	0.49	0.32	0.43
	1/2000 Mol. KNC (Two Hrs. Before Test).			1/5000 Mol. KNC (Two Hrs. Before).			1/10000 Mol. KNC (One Hr. Before).		
First hour KNC	0.20	0.13	0.23	0.23	0.16	0.27	0.41	0.24	0.34
Second hour KNC	0.14	0.16	0.21	0.14	0.16	0.22	0.41	0.22	0.33
Per cent. decrease	59	64	58	56	56	45	15	32	23
Recovery	0.34	0.48	0.50	0.48	0.34	0.43			

TABLE IV.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Arenicola cristata.*

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Aug. 2, 20.5° C.			Aug. 1, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.42	0.39	0.67	0.42	0.48	0.43
Second hour normal	0.34	0.38	0.63	0.44	0.47	0.56
	1/1000 Mol. KNC (Two Hrs. Before).			1/2000 Mol. KNG (Two Hrs. Before).		
First hour KNC	0.27	0.30	0.46		0.22	0.31
Second hour KNC	0.20	0.26	0.46	0.27		0.30
Per cent. decrease	39	28	30	35	54	39
Recovery	0.49	0.31	0.71	0.42	0.48	0.43

solutions of cyanide into these animals, presumably because of their thick body walls, the practice of leaving them for an hour or two in the cyanide solution before carrying out the test was adopted.

The results with *Chætopterus* are presented in Table III., with *Arenicola* in Table IV, and with *Nereis* in Table V. One or two individuals, depending on size, were placed in each flask. For reasons already given, experiments 1–3, Table V., on *Nereis* were not very satisfactory but the other experiments on these polychætes leave no doubt that cyanide brings about a reversible decrease in oxygen consumption.

TABLE V.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Nereis virens*.

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Aug. 5, 22° C.			Aug. 6, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.80	0.65	0.65	0.30	0.36	0.29
Second hour normal	0.78	0.55	0.64	0.23	0.23	
	1/5000 Mol. KNC (Two Hrs. Before Test).			1/1000 Mol. KNC (One-half Hour Before).		
First hour KNC	0.14	0.11	0.11	0.05	0.05	0.08
Per cent decrease	83	82	83	83	84	73
Recovery	0.32[1]		0.48[1]	0.34		0.29

EXPERIMENTS ON LEECHES.

The leeches used in these experiments were *Hæmopis marmoratis*, and *Herpobdella punctata*, chiefly the latter. They were obtained from ditches near Wolf Lake, Indiana, and subjected to experiment shortly after they were brought into the laboratory. Two or three individuals were placed in each flask. As in the case of the polychætes, it was impossible to eliminate movement, but the degree of activity was about the same in both normal and cyanide-containing water. The results are given in Table VI. Experiments 4 and 7 were performed upon *Hæmopis*, the others on *Herpobdella*.

[1] Failure to recover original rate of oxygen consumption not due to cyanide, see text.

TABLE VI.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF LEECHES (*Hæmopis marmoratis*, EXPS. 4 AND 7, AND *Herpobdella punctata*, REMAINING EXPS.).

No. of Experiment	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Temp. and Date	April 3, 21° C.			Mar. 22, 21° C.			April 1, 21° C.			
Oxygen Consumed, Cubic Centimeters per Hour.										
First hour normal	0.15	0.22	0.19	0.51	0.31	0.28	0.51	0.21	0.24	0.20
Second hour normal	0.16	0.24		0.51	0.30	0.25	0.48			
	1/5000 Mol. KNC.			1/10000 Mol. KNC.			1/25000 Mol. KNC.			
First hour KNC	0.01	0.07	0.04	0.17	0.17	0.15	0.29	0.08	0.17	0.14
Second hour KNC	0.04	0.07	0.06	0.13	0.14	0.11	0.26	0.09	0.14	0.13
Per cent. decrease	84	70	74	73	51	51	45	60	36	33
Recovery	0.17	0.28	0.23		0.26	0.33		0.16	0.23	0.19

EXPERIMENTS ON OLIGOCHÆTES.

The forms used in these experiments were an aquatic earthworm, *Helodrilus tetædra*, one of the megadrilous oligochætes, and *Lumbriculus inconstans*, one of the microdrilous oligochætes. Both of these annelids live in temporary pools in the woods in the dune region of Indiana; the former occurs in the larger pools and also in permanent ponds, while the latter species has never been found in permanent bodies of water. They were collected near Clarke, Indiana. Both species are highly thigmotactic, being found in their natural habitat entwined among the branches of the moss which commonly grows in these pools or lying between layers of dead leaves, their posterior ends usually protruded for respiratory purposes. As the animals are very restless when their bodies are not in contact with objects, an attempt was made to quiet them by placing a small amount of thoroughly washed cotton in the experimental flasks with the worms for some hours preceding the tests. This procedure was entirely successful with *Helodrilus;* the worms entwined themselves among the cotton fibers and remained perfectly quiet throughout the experiments. It was possible to quiet the majority of the individuals of *Lumbriculus* in this way also, but a few individuals would always continue to crawl about. No difference, however,

was observed in the degree of activity in normal and in cyanide-containing water.

The results with *Helodrilus* are presented in Table VII. and with *Lumbriculus* in Table VIII. As these worms are rather

TABLE VII.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Helodrilus tetædra.*

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	June 3, 23° C.			June 2, 23° C.		
Oxygen Consumed, Cubic Centimeters in Two Hours.						
First hour normal	0.13	0.16	0.16	0.17	0.18	0.19
Second hour normal	0.13	0.19	0.15	0.16	0.22	0.22
	1/5000 Mol. KNC.			1/10000 Mol. KNC.		
First hour KNC	0.05	0.08	0.09	0.13	0.16	0.11
Second hour KNC	0.06	0.06	0.07	0.09	0.12	0.08
Per cent. decrease	59	60	49	30	30	54
Recovery	0.14	0.16		0.14	0.19	0.16

TABLE VIII.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Lumbriculus inconstans.*

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Oct. 17, 21° C.			Oct. 15, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.44	0.47	0.37	0.58	0.58	0.46
Second hour normal	0.45	0.41	0.29(?)	0.57	0.61	0.49
	1/5000 Mol. KNC.			1/10000 Mol. KNC.		
First hour KNC	0.20	0.24	0.18	0.30	0.28	0.21
Second hour KNC	0.14	0.16	0.14	0.28	0.26	0.19
Per cent. decrease	62	55	52	50	55	56
Recovery	0.38	0.42	0.42			

small, a large number of individuals was used in each experiment. The results are the same as in the case of the other animals tested, a reversible decrease in oxygen intake when cyanide is present.

Summary and Conclusions.

1. The normal oxygen consumption and the oxygen consumption in the presence of various concentrations of potassium cyanide was tested in the case of *Pennaria*, *Metridium*, *Nereis*, *Chætopterus*, *Arenicola*, two species of leeches, and two species of aquatic oligochætes. In all cases, numbering about seventy experiments (not all of which are reported), the oxygen consumption was markedly decreased in the presence of cyanide.

2. This decrease was not due to differences in muscular activity, since in some of the experiments it was possible to keep the animals entirely quiet throughout, while in the others, with three exceptions noted in the text, the animals were equally active in both the normal and the cyanide-containing water. None of the concentrations of cyanide used caused any visible anæsthesia, within the short time periods during which the animals were exposed to them.

3. The decrease was reversible, the oxygen consumption returning to approximately the original value when the cyanide was washed out of the animals. In no case were the animals injured in any way. Most of them were kept for a considerable length of time after the experiments were completed and were entirely normal in behavior and appearance.

4. As was found to be the case in previous experiments with cyanide, the percentage of decrease is absolutely greater, the more concentrated the cyanide solution, but the more dilute solutions are relatively more effective.

5. These results are in accord with previous experiments on the action of cyanides and justify the use of the cyanides as depressing agents.

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